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# MAIN MICROBIOLOGICAL POLLUTANTS OF BOTTLED WATERS AND BEVERAGES

**Carmen Curutiu<sup>\*,†</sup>, Florin Iordache<sup>‡,§</sup>, Petruta Gurban<sup>¶</sup>,  
Veronica Lazar<sup>\*,†</sup>, Mariana Carmen Chifiriuc<sup>\*,†</sup>**

*\*Faculty of Biology, University of Bucharest, Bucharest, Romania †Research Institute of the University of Bucharest (ICUB), Bucharest, Romania ‡Institute of Cellular Biology and Pathology Nicolae Simionescu, Bucharest, Romania*

*§Department of Biochemistry, University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania ¶Stefan S. Nicolau Institute of Virology of Romanian Academy, Bucharest, Romania*

## 14.1 Introduction

Water is essential for every form of life, being necessary for all biological processes. At the global level, water is a limited resource, fact which requires care in its use for a sustainable preservation of water resources. Clean, safe, and hygienic water are other aspects that people take care for assuring public health. The consumption of water of inadequate quality could lead to high rates of waterborne disease. The quality of tap water is not always the best, reason for which in the last years, consumption of bottled water has increased. But although people perceive the bottled water being clean and safe, this is not always the truth, the bottled water being also chemically or microbiologically contaminated, raising risks for diseases (Khaniki et al., 2010).

Because the isolation of pathogenic microorganisms from the water involves a complex and costly analysis as well as the fact that their presence in water is not always constant, in practice an indirect method of highlighting their potential presence is used, involving the determination of some microbiological indicator species, whose presence in water signals the risk of fecal pollution and implicitly the risk of contamination with pathogenic microorganisms. In general, the following indicator parameters are used to determine the degree of microbiological contamination of water: total number of microorganisms grow at 22°C; total number of microorganisms grow at 37°C; total coliform bacteria (total coliforms), fecal coliforms; the presence of *Escherichia coli*; fecal streptococci (enterococci).

In special situations (new water sources, control of water treatment plants, accidental pollution, water epidemics, etc.) the following parameters are also determined: the presence of sulfate reducing *Clostridium* species and the presence of *Clostridium perfringens*; the presence of *Pseudomonas aeruginosa*; the presence *Salmonella* species; enteric bacteriophages (Chifiriuc et al., 2016). It was proved that none of these established tests is absolute, because sample of water although consistent with these requirements could be at the origin of some infectious diseases. For example, samples negative for *E. coli* indicator can be contaminated with viruses or protozoa like *Cryptosporidium* spp. (Edberg et al., 2000).

## 14.2 Main Microbiological Pollutants of Bottled Waters

Drinking water contaminants can be classified in physical, chemical, biological, or radiological substances or matter. Some of these contaminants may be harmful for the animal and human organisms, while others may be harmless. Biological contaminants are represented by bacteria, protozoa, yeast, fungus, and toxins produced by these organisms as well as viruses that are responsible of different diseases in humans, most frequently gastroenteritis (Table 14.1).

**Table 14.1 The Major Types of Microbiological and Viral Contaminants of Water and the Associated Diseases**

Type of Contaminant	Species	Type of Infection
Bacteria	<i>Campylobacter jejuni</i> , <i>Campylobacter coli</i>	Mild self-limiting gastrointestinal illness
	<i>Escherichia coli</i>	Gastrointestinal illness and kidney failure
	<i>Helicobacter pylori</i>	Capable of colonizing human gut that can cause ulcers and cancer
	<i>Legionella pneumophila</i>	Lung diseases when inhaled
	<i>Mycobacterium</i> spp.	Lung infection in those with underlying lung disease, and disseminated infection in the severely immunocompromised persons
	<i>Shigella sonnei</i>	Mild self-limiting gastrointestinal illness and bloody diarrhea

**Table 14.1 The Major Types of Microbiological and Viral Contaminants of Water and the Associated Diseases—cont'd**

Type of Contaminant	Species	Type of Infection
Viruses	<i>Salmonella enterica</i>	Mild self-limiting gastrointestinal illness
	<i>Vibrio cholera</i>	Gastrointestinal illness, watery diarrhea
	<i>Pseudomonas aeruginosa</i>	Hospital-acquired infections
	<i>Klebsiella pneumoniae</i>	Pneumonia, typically in the form of bronchopneumonia
	<i>Aeromonas hydrophila</i>	Gastroenteritis, septicemia, traumatic and aquatic wound infections, and infections after medical leech therapy
	<i>Enterobacter cloacae</i>	Gastrointestinal illness
	Adenovirus	Respiratory illness and occasionally gastrointestinal illness
	Astrovirus	Gastroenteritis in children
	Caliciviruses (rotaviruses, noroviruses)	Mild self-limiting gastrointestinal illness
	Enterovirus (polioviruses, coxsackie viruses, echoviruses)	Mild respiratory illness
Protozoans	Hepatitis A and E viruses	Liver disease and jaundice
	<i>Cryptosporidium</i> spp.	Enteric disease
	<i>Giardia lamblia</i>	Intestinal disease, diarrhea among children
Yeasts and fungi	<i>Naegleria fowleri</i>	Primary amebic meningoencephalitis
	<i>Penicillium</i> spp., <i>Penicillium citrinum</i> , <i>P. glabrum</i>	
	<i>Aspergillus</i> spp.	
	<i>Candida</i> spp., <i>Candida glabrata</i> , and <i>Candida albicans</i>	
	<i>Cladosporium</i> spp., <i>Cladosporium cladosporioides</i>	
	<i>Alternaria alternate</i>	
	<i>Rhizopus</i> spp.	
	<i>Fusarium</i> spp.	
	<i>Trichoderma viride</i>	
	<i>Aspergillus</i> spp.	
Toxins	<i>Phoma</i> spp.	
	<i>Phialophora richardsiae</i>	
	Geosmin ( <i>Chaetomium globosum</i> )	
	Aflatoxins ( <i>Aspergillus</i> spp.)	
	Patulin ( <i>Aspergillus</i> spp., <i>Penicillium</i> spp., <i>Byssosclamyces</i> spp.)	
	Zearalenone ( <i>Fusarium</i> spp.)	
	Fumonisin ( <i>Fusarium</i> spp.)	
	Neosolaniol ( <i>Fusarium</i> spp.)	
Ochratoxin ( <i>Aspergillus</i> spp., <i>Penicillium</i> spp.)		

### 14.2.1 Main Bacterial Contaminants

Coliform bacteria in bottled water represent a great threat to public health, especially for infants, young children, and immunocompromised persons that could contact waterborne diseases, even at lower infectious doses. World Health Organization (WHO) recommends that potable water should have below 20 CFU/mL heterotrophic bacterial counts with no coliform bacteria, fecal coliforms, *E. coli*, enterococci, and *P. aeruginosa* (Khatoon and Pirzada, 2010). Coliform bacteria belong to *Enterobacteriaceae* family and include species of the following genera: *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Serratia*, and *Yersinia*. The presence of coliform microorganisms in drinking water represents a sign of fecal contamination and indicates the potential contamination also with pathogenic bacterial species such as *Shigella* spp., *Salmonella* spp., or *Vibrio cholerae*. They are used traditionally as indicators of water quality because the testing methodology is simple and inexpensive.

*E. coli* can survive in drinking water at 15–18°C for 4–12 weeks and does not multiply significantly in the external environment. According to WHO (2006) *E. coli* are rarely found in water in the absence of fecal pollution, and no differentiation is made between pathogenic and nonpathogenic *E. coli* when this species is isolated from water. Therefore, water that contains *E. coli* is unsafe for consumption due to the strong association between *E. coli* and fecal contamination.

At least six different main groups of *E. coli* were differentiated, based on the specific virulence factors of the strains and the clinical features of the disease, from which enterotoxigenic (ETEC), enterohemorrhagic (EHEC), and enteroinvasive (EIEC) serotypes are most harmful and could be transmitted by contaminated water (Bettelheim, 2003; Scheutz and Strockbine, 2005).

ETEC serotype can cause infantile gastroenteritis. Intense watery diarrhea that lasts several days, dehydration and malnutrition in young children represents some of the manifestation of the disease. Although in developed countries the number of the cases is small, in the developing country, because of an adequate clean water and poor sanitation, the reports indicate that this serotype is an important cause of diarrhea, being the most frequently isolated bacterial enteric pathogen in children under 5 years of age. This serotype is also known as the main common cause of “travelers’ diarrhea” at people from developed countries who travel in these developing regions.

EHEC serotype produces Shiga-like toxins and determines abdominal pain, bloody diarrhea, and hemolytic uremic syndrome. The symptoms last 7–10 days, but in some cases, infections result in acute renal failure. Although EHEC strains are not ordinarily contaminants of treated drinking water, some reports indicate that some drinking water sources contaminated with human sewage or cattle feces. Some

authors reported that EHEC strains could survive some months in manure and water-trough sediments, isolated from different sources of water such as ponds or streams, wells, and water troughs.

EIEC strains determine gastroenteritis, enterocolitis, and dysentery accompanied by well-known symptoms as abdominal cramps, diarrhea, emesis, febrility, chills, a generalized malaise, and stools with blood and mucus. Similar with *Shigella*, they are invasive species that can enter and multiply in the intestinal epithelial cells of the distal large bowel in humans (Cabral, 2010).

*Campylobacter* spp. infections together with *Salmonella* spp. and *Shigella* spp. infections are the leading cause of acute gastroenteritis worldwide (Frost, 2001). The main sources of contamination with *Campylobacter* spp. are consumption of raw meat, untreated drinking water, raw milk, and the presence of pets (Brown et al., 2004; Friedman et al., 2004). Also, the most common species isolated in developed and developing countries are *Campylobacter jejuni* and *Campylobacter coli*. Their presence in water is due to wastewater discharge and farming activities (Evans et al., 2003). Survival of *Campylobacter* in different type of waters (ground, surface, and drinking waters) is conditioned by a variety of factors such as temperature, nutrients, water microbiota, and biotic interactions (Talibart et al., 2000; Cools et al., 2003). A study by Tatchou-Nyamsi-König et al. (2007) whose objective was to evaluate *C. jejuni* survival of at 4°C and 25°C in filtered natural mineral water proved that the microaerophilic *C. jejuni* survive better at temperature around 4°C, high temperatures (16–22°C) affecting the cell viability. The study also demonstrated that even in the presence of biodegradable organic matter the bacteria could survive and multiply for a relatively long time, despite of the presence of oxygen, especially at low temperature (Tatchou-Nyamsi-König et al., 2007).

Other contaminants of bottled water could be represented by *Enterococcus* species that are Gram-positive cocci facultative anaerobes displaying alkaline and salt tolerance. Genus *Enterococcus* formerly referred to as the fecal streptococci or Lancefield group D comprises many species of which *Enterococcus faecalis* and *Enterococcus faecium* are predominant. Most enterococci do not multiply in water but can survive in water longer than coliforms and most enteric pathogens. The 2006 WHO drinking water report showed that enterococci are indicators of chronic fecal pollution serving as a marker for fecal pathogens that survive longer in water than *E. coli* (WHO, 2006). Water containing enterococci should be considered unsafe due to the strong association between enterococci and fecal contamination (Report of the Scientific Committee of the Food Safety Authority of Ireland, 2009).

Pseudomonads found in soils, ground water, marine environments, plants, are part of the normal microbiota of bottled water. *P. aeruginosa* is Gram-negative opportunistic bacteria which are

responsible of a wide range of infections especially in patients with compromised host defense mechanisms (Zamberlan da Silva et al., 2008). The presence of *P. aeruginosa* causes urinary and respiratory tract infections, in patients who are severely immunocompromised (e.g., patients with neutropaenia, cystic fibrosis, and severe burns), being difficult to eradicate due to its high intrinsic resistance and ability to transfer its resistance genes. *P. aeruginosa* requires a high oral infectious dose, of minimum 1.5 million bacteria to cause an infection and normally *P. aeruginosa* does not infect the healthy tissue. *P. aeruginosa* cannot grow and survive in natural mineral water, and when detected, it is in low numbers (Leclerc and Moreau, 2002). Edberg et al. (1996) conducted a study in which they determined the numbers and types of bacteria found in three water sources (bottled water, water cooler water, and tap water). They took samples from a wide variety of water types and determined the presence of heterotrophic plate count (HPC) bacteria, total coliforms, and *P. aeruginosa*. They also determined the virulence factors expression, susceptibility to antibiotics, acid lability, and cytotoxicity testing using Hep-2 cells. Their results showed that all water sources presented a normal bacterial content, and only 2% of bottled water samples were positive for *P. aeruginosa*.

Vantarakis et al. (2006) investigated the microbiological quality of over 1000 samples of bottled non-carbonated (“still”) mineral water, from more producing companies in Greece, during the period 1995–2003. Around 14% of the samples were inadequate according to Greek water regulation. The genera identified from the samples were *Pseudomonas*, *Aeromonas*, *Pasteurella*, *Citrobacter*, *Flavobacterium*, *Providencia*, and *Enterococcus*, the species *P. aeruginosa* being the most frequently isolated.

Another study from Puerto Rico identified as main bacterial contaminants from commercial bottled waters species as *Pseudomonas fluorescens*, *Corynebacterium* sp. J-K, *S. paucimobilis*, *Pseudomonas vesicularis*, *Aeromonas baumannii*, *Pseudomonas chlororaphis*, *Flavobacterium indologenes*, *Aeromonas faecalis*, and *Pseudomonas cepacia* (Reyes et al., 2008).

*Helicobacter (H.) pylori* is a Gram-negative, microaerophilic bacterium involved in the development of gastric adenocarcinoma, gastritis, and mucosa-associated lymphoid tissue lymphoma. Furthermore, *H. pylori* are the primary cause of ulcers in the stomach and duodenum. More than 50% of world populations have been infected with *H. pylori*, but exact routes of transmission are still unknown, contaminated water playing an important role in spreading of *H. pylori* to humans (Ranjbar et al., 2016).

*Legionella pneumophila* is an aerobic, pleomorphic, flagellated, nonspore-forming, and Gram-negative bacterium. In a study that

used 68 commercial bottled mineral waters were identified *Legionella* antigen in eight samples (confirmed by PCR as *L. pneumophila*), but were negative in cultures. Whether the bacteria are alive or dead the risk for infection is high especially for the immunocompromised patients (Klont et al., 2006).

*Mycobacterium (M.) tuberculosis* is a pathogenic bacteria from *Mycobacteriaceae* family. *M. tuberculosis* is part of a complex that contains at least nine species: *M. tuberculosis* sensu stricto, *M. africanum*, *M. canetti*, *M. bovis*, *M. caprae*, *M. microti*, *M. pinnipedii*, *M. mungi*, and *M. orygis*. Until 1882 *M. tuberculosis* was the only clinically significant species, then numerous reports led to the discovery of atypical nontuberculous mycobacteria (NTM). NTM can survive, persist, and develop in drinking water supply systems. Recent data disclose that water may be the transporter for these mycobacteria that infect or colonize the host. NTM species can cause opportunistic infections in immunocompromised patients. In AIDS the major mycobacteria species and the most common cause of death is *Mycobacterium avium*. In an US study focusing on *M. avium* and *M. intracellulare* in drinking water showed that these mycobacteria can be clinically relevant in human population. From 139 cisterns, noncarbonated bottled waters, or reservoir samples, 46 (33%) were positive for NTM. The most frequently NTM species isolated from water are: *M. kansasii*, *M. mucogenicum*, and *M. peregrinum* (Covert et al., 1999).

## 14.2.2 Viruses

More than 140 enteric viruses can infect humans and are considered to be emerging waterborne pathogens because of their high stability in the environment and resistance to current water treatment processes. HEntVs include families: (i) *Picornaviridae* (polioviruses, enteroviruses, coxsackieviruses, echoviruses, and hepatitis A virus), (ii) *Caliciviridae* (noroviruses and sapoviruses), (iii) *Astroviridae* (astroviruses), (iv) *Reoviridae* (rotaviruses), (v) *Hepeviridae* (hepatitis E virus), and (vi) *Adenoviridae* (adenoviruses) (Fong and Lipp, 2005). These viruses are associated with mild respiratory illness, gastroenteritis, but also encephalitis, meningitis, myocarditis (enteroviruses), cancer (polyomavirus), and hepatitis (hepatitis A and E viruses). Other viruses such as polyomaviruses and cytomegalovirus influenza and coronaviruses can potentially be spread through water but evidence is not clear (WHO, 2011). Viral infections are in the most cases self-limiting in healthy individuals, but can cause higher rates of morbidity in children under the 5 years old, in elderly, immunocompromised people, and pregnant women. In developing countries, waterborne virus-based diseases present higher level because widespread malnutrition and numerous HIV-positive people (Gall et al., 2015).

Contamination of drinking water is supported by (i) low infectious doses of viruses; (ii) persistence long periods of time in water; and (iii) very high numbers of viral particles in feces. Also, drinking water can transport viruses through inhalation (e.g., showering) or by direct contact with eyes or skin (e.g., swimming). The United States Environmental Protection Agency (USEPA) is assessing adenovirus, enteroviruses, caliciviruses, and hepatitis A virus for potential regulatory action (U.S. EPA, 2009). The current regulations involve the removal or inactivation of 99.99% of enteric viruses using approved techniques. Viruses are small particles and conventional treatment, including filtration is usually ineffective, so the most viruses are inactivated using oxidants. Water treatment techniques include physically removing viruses through conventional treatment and inactivating viruses using ultraviolet light or chemical substances such as chloramines, chlorine dioxide, chlorine, and ozone.

The presence of viruses is not usually on the list of the main indicators for microbiologic contamination in bottled water, because detection of viruses from drinking water is more difficult in comparison to detection of bacteria. Viral multiplication requires the use of tissue culture, involving time, expertise, labor, and expensive equipment. Furthermore, some of these viruses are difficult (e.g., adenovirus serotypes 40 and 41) or impossible (human norovirus, hepatitis A virus) to cultivate in cell cultures. Molecular biology techniques such as ELISA and PCR allow a rapid analysis of viruses, but they do not distinguish between particles with infectious properties versus particles with non-infectious properties.

It has been estimated that 30%–90% of waterborne disease outbreaks worldwide are caused by human *enteric viruses* (HEntVs). HEntVs are transmitted primarily by the fecal-oral path, either direct from person-to-person or via ingestion of contaminated food or water.

The human adenovirus (HAdV) belongs to the *Adenoviridae* family and can be a potential marker of human fecal contamination in water. Enteric viruses are more resistant to environmental conditions and water treatment by chlorination and filtration than enteropathogenic bacteria. In water there is no potential for replication because the viruses are obligate intracellular parasites, but however, the viral degradation is slower than that of bacteria in water and the amount of remaining viruses has a significant potential for starting an outbreak (Nascimento et al., 2015).

Choi and Jiang (2005) showed that 16% of the river samples in California, United States were positive for HAdV. Also, Albinana-Gimenez et al. (2009) found the HAdV in river water samples in Barcelona. Furthermore, Dong et al. (2010) detected the HAdV in sewage and recreational water samples from New Zealand. Wyn-Jones et al. (2011) showed that European recreational and fresh water

samples were positive for HadV (60.6%). In China, [Ye et al. \(2012\)](#) described 100% the presence of HAdV in river and drinking water samples.

Both *hepatitis A and E* are reported worldwide. Hepatitis A (HEA) is particularly frequent in countries with poor sanitary and hygienic conditions (in Africa, Asia, and Central and South America). Countries with economies in transition and some regions of industrialized countries where sanitary conditions are under-standard and are also highly affected (southern and eastern Europe and some parts of the Middle East). In recent years, some outbreaks have occurred in areas of conflict and emergencies, such as war zones, and in sites for refugees.

An estimated 20 million infections and 3.3 million symptomatic cases of hepatitis E (HEV) occur annually worldwide with a number of deaths estimated at 56,000. Cases and outbreaks of this disease often go undiagnosed or misdiagnose, confused with other forms of hepatitis because of the similarity of this disease with other forms of acute viral hepatitis, and limited availability and use of specific diagnose tests for it. The incubation period of hepatitis E varies from 2 to 10 weeks, with most cases occurring 4–6 weeks after exposure. Hepatitis E virus has a 7.2-kb single-stranded RNA genome that forms 27–34 nm non-enveloped virions. The virus has at least four distinct genotypes, 1 and 2 infect only humans, and are responsible for the majority of human diseases caused by infection with this virus, and genotypes 3 and 4 are found among mammalian animals, including pigs, wild boar, deer, and only occasionally infect humans. Genotypes 1 and 2 have been associated with large waterborne outbreaks, and genotype 3 has been identified in occasional small foodborne outbreaks reported from developed countries. Heating to 60°C for a few minutes has been shown to inactivate a large proportion of HEV particles suggesting that drinking water can be rendered safe by pasteurization or boiling. Several epidemics of hepatitis E have been found to be related to the failure of chlorination, suggesting that chlorine treatment protects against hepatitis E. Thus, although there is no direct evidence that chlorine inactivates HEV, chlorination of drinking water with adequate residual chlorine levels at the point of consumption continues to be a good public health intervention ([De Serres et al., 1999](#)).

*Norovirus* epidemics associated with drinking water are reduced in developed countries, although it was implicated in a waterborne outbreak in Spain. More than 4000 cases of gastroenteritis were detected during April 11–25, 2016, the patients presenting symptoms such as vomiting, diarrhea, nausea, abdominal pain, or fever. The epidemiologic investigation revealed a correlation between the outbreak and drinking bottled spring water from office water coolers. The contaminated samples fulfilled all the other requirements for natural mineral

water. The company producing these waters recalled anyway more than 6000 containers of suspected water as a safety measure (Albert et al., 2017).

### 14.2.3 Protozoan Drinking Water Contaminants

Water is also an important vehicle of many parasitic diseases. *Cryptosporidium*, an emerging pathogen, is a major cause of enteric infections in humans and animals. Cryptosporidiosis can be severe especially in children, elderly people, and malnourished persons and life-threatening in those whose immune systems is depressed. In AIDS patients, infections with *Cryptosporidium* are lethal, no drug therapy has been found to be effective (Fayer et al., 2000). The oocysts are transmitted either directly, by host-to-host contact or indirectly, by drinking contaminated water or consumption of contaminated food (Franco and Neto, 2002).

Recent data showed that cysts are resistant in 100% chlorine for 48 h. The shell of the cyst is disintegrated by the intestinal content of animals and humans. Therefore, elimination of cysts from water can be done by filtration.

In water, *Cryptosporidium* is found in a cyst form, protected by a shell making it resistant to traditional disinfectants. In the intestinal tract, the cyst walls are destroyed, releasing the parasite that multiply into the body and provoke symptoms such as cramps, diarrhea, or even death. In 1993 in Wisconsin over 400,000 persons suffered from gastrointestinal illness when a contamination with *Cryptosporidium* was found in the public water supply, and over 100 immunocompromised people died. More recently in other cities such as New York City, Nevada, Oregon, and Georgia was reported the presence of *Cryptosporidium* in water. Specialists estimate that ~50% of the city water supplies may have *Cryptosporidium* present but most cities have not yet been tested.

*Giardia lamblia* is a flagellated protozoan that causes significant intestinal diseases in both humans and animals. Epidemiological features of *Giardia* are similar with *Cryptosporidium*, representing one of the major public health concerns regarding water utilities today. *Giardia* is a reemerging infectious agent due to high number of outbreaks of diarrhea among children (Thompson, 2000). In Brazil the level of contamination of ground and raw water with *Cryptosporidium* and *Giardia* is very high, and also bottled mineral water was tested. *Giardia* cysts can be eliminated from water by a filtration method using filters that retain all particles of at least 4  $\mu\text{m}$ . In Mexico, three brands of mineral water, the protozoan was found using membrane filtration and in vitro culture tests (Nichols et al., 2004).

Other protozoa species identified in water samples are the non-pathogenic amoebae *Vahlkampfia vahlkampfia*, *Naegleria gruberi*, and *Acanthamoeba astronyxis* and the flagellate *Bodonomorpha minima*. In France, a nonpathogenic amoebic species was detected in mineral waters originating from small-capacity regional plants. Franco and Neto detected 13 *Cryptosporidium* oocysts by membrane filtration followed by immunofluorescence in 2 of 13 bottled water samples.

*Naegleria* is another amoeba commonly found in warm freshwater (lakes, rivers, and hot springs) and soil. The amoeba can be found also in swimming pools that are poorly maintained, minimally chlorinated, and/or unchlorinated, in water heaters because *Naegleria fowleri* grows best at temperatures up to 115°F (46°C) and also in salt water, like the ocean (CDC). Only one species of *Naegleria*, that is, *Naegleria fowleri* infects people. The incidence of infection with *Naegleria fowleri* is very low, only 40 cases being reported in the United States in the 10 years from 2007 to 2016. The majority of infections have been caused by *Naegleria fowleri* from freshwater located in southern-tier states.

#### 14.2.4 Yeasts and Fungi

*Candida* (*C.*) spp. are the most prevalent yeasts isolates from the water samples. There are now at least 17 species of *Candida* with medical importance, some of them being found in water samples: *C. parapsilosis*, *C. glabrata*, *C. albicans*, *C. tropicalis*, and *C. guilliermondii*. The incidence of nosocomial infections caused by *Candida* strains is growing due to changes of the implicated species, antibiotic resistance, and host-related predisposing factors. Some studies reported the presence of fungal biofilms in municipal water distribution systems. Fungi established in biofilms in water systems are more resistant to water treatment, and can colonize filters in treatment plants, and consequently affect the water treatment. The ecology of fungi in biofilms has been studied only to a small degree, and further research should aim to investigate the features of fungi grown in biofilms (Gunhild et al., 2009).

In the literature, few reports are regarding the occurrence of yeasts and fungi in treated and bottled mineral waters (Yamaguchi et al., 2007).

From filamentous fungi, *Penicillium* (*P.*) spp., *Penicillium citrinum*, *Penicillium glabrum*, *Cladosporium cladosporioides*, *Alternaria alternate*, *Cladosporium*, *Rhizopus*, *Aspergillus*, and *Phoma* were the most frequently isolated species. Some of these are potentially pathogenic, allergenic, and toxigenic species.

Cabral and Pinto (2002) reported the presence of filamentous fungi in more the 100 samples of still bottled mineral water produce by different commercial brands (eight) from Argentina.

In a study in 2001 *Aspergillus fumigatus* was found in tap water from Rikshospitalet University Hospital in Oslo. *Aspergillus fumigatus* is a fungal pathogen causing infections at immunocompromised patients, suggesting that hospital water systems may serve as a transmission path for fungal infections. These data indicate that hospital water may contain also other species of fungi, including potential pathogens. A hypothesis is that fungi in water are aerosolized into air when water passes the system, such as taps and showers, reaching to immunocompromised patients. Air levels of *Fusarium* and *Aspergillus* were found to increase in hospital environments after running showers multiple times.

When it comes to the allergenic potential of fungi in water, three cases have been reported from Sweden and Finland. An outbreak with *Phialophora richardsiae* in water was reported. Two case reports from Finland indicated fungal contaminated water as the source of hypersensitivity pneumonitis. *Trichoderma viride* was the most dominant species in Norwegian drinking water and has been associated with asthma in children living in water-damaged homes.

### 14.2.5 Main Microbial Toxins Found in Water

The allergic potential of fungi in drinking water is not fully documented, large epidemiological studies being needed to investigate the presence of different toxins. Several microorganisms can cause taste and odor problems in drinking water. Several fungal species, such as, for example, *Chaetomium globosum* have been found to produce geosmin, a compound related with earthy odor and taste in drinking water. In addition, fungi may produce a variety of other compounds with distinctive off-flavors and tastes.

Mycotoxins are secondary metabolites produced by some fungi species. Exposure of humans or animals to them can cause serious health problems, some of them are considered carcinogenic. Such exposure is likely to occur from dietary intake of contaminated food, water, or beverages. Therefore, actions to prevent mycotoxin contamination of water have began in water treatment plants, consisting in first filtering the water and finally add adequate disinfectants to remove or mitigate fungi or their toxic metabolites. Several of the species of *Penicillium* and *Aspergillus* are known to produce mycotoxins in other substrates, such as food and beverages. Some fungi, including *Penicillium* spp., *Aspergillus* spp., *Fusarium* spp., and *Claviceps* spp. are known to produce mycotoxins such as patulin, aflatoxins, and zearalenone. Toxins such as: aflatoxins, ochratoxin, fumonisins, and neosolaniol were identified by LC-MS/MS (Al-Gabr et al., 2014; Ribeiro et al., 2006).

Cyanobacteria (*Anabaena*, *Microcystis*, *Clyndospermopsis nodularis*) produce water blooms that increase the level of toxins. They can act as hepatotoxins and neurotoxins, and can kill animals (goats, sheep, horses, pigs, dogs) within minutes or hours of water ingestion from these lakes. Toxins produced by cyanobacteria may also have mutagenic effects. Annually these toxins produce 100,000–200,000 of serious illnesses with neurological sequelae/10,000–20,000 deaths.

Algae also synthesize biotoxins, causing frequent intoxications by ingestion (diarrhea, neurotoxic phenomena) in tropical/subtropical areas, generating serious neurological sequelae (amnesia, paralysis). Microcystin is 1000 times more toxic than cyanide. Annually, 10,000–50,000 cases/year of ciguatera toxin poisoning are reported. Algal toxins are relatively stable in the dark and can last for at least 1 week in water (Chifiriuc et al., 2016).

## 14.3 Main Microbial Contaminants of Beverages

The contamination of microbial beverages occurs during fabrication process. Microbial problems of the beverages can be separated in two categories: (i) the spoilage of the product due to the growth of the microorganism in the juice that causes its deterioration, making it unpleasant for human consumption; and (ii) the contamination of the product due to the growth of microorganism determines food poisoning. The raw materials but also the environment conditions are the risk factors.

Soft drinks are high in water activity and often rich in vitamins and minerals, so they represent a good growth medium for different microorganisms. On the other hand due to the acidity of the medium, a few microorganisms can survive and multiply. Aciduric microorganisms are the main contaminants of the bottled beverages: yeasts, bacteria, molds, and mycotoxins (Table 14.2).

### 14.3.1 Yeasts

Yeasts are the main microbial contaminants of beverages, due to their capacity to survive in an acidic environment and at high carbonation levels. These species have an optimal pH value at 3.0–6.5, but are able to survive from pH 1.5–8.5. *Zygosaccharomyces bailii*, *Saccharomyces*, *Brettanomyces*, *Hanseniaspora*, *Hansenula*, and *Pichia* are the most encountered species founded in beverages involved in spoilage (Lawlor et al., 2009; Sperber, 2009). The process of spoilage could have many aspects: (i) the beverages become

## Table 14.2 The Main Microbial Contaminants of Beverages

Type of Contaminant	Species	Type of Contamination
Yeasts	<i>Zygosaccharomyces bailii</i> <i>Saccharomyces</i> <i>Brettanomyces</i> <i>Hanseniaspora</i> <i>Hansenula</i> <i>Candida davenportii</i> <i>Candida parapsilosis</i> <i>Pichia</i> <i>Debaryomyces</i> spp. <i>Rhodotorula</i> <i>Sporidiobolus</i> <i>Sporobolomyces</i> <i>Aureobasidium</i>	Beverage spoilage
Molds	<i>Aspergillus</i> <i>Penicillium</i> <i>Rhizopus</i> <i>Cladosporium</i> <i>Alternaria</i> <i>Fusarium</i> <i>Byssochlamys spectabilis</i> <i>Talaromyces</i> spp. <i>Penicillium arenicola</i> <i>Neosartorya</i> spp.	Beverage spoilage
Bacteria	Lactic acid bacteria (LAB) <i>Lactobacillus</i> ( <i>L.</i> ) <i>paracasei</i> , <i>L. brevis</i> , <i>L. buchneri</i> , <i>L. plantarum</i> , <i>L. perolens</i> , <i>Leuconostoc mesenteroides</i> , <i>Weissella confuse</i> spp. Acetic acid bacteria (AAB)- ( <i>Acetobacter</i> , <i>Gluconobacter</i> , <i>Gluconacetobacter</i> ) Coliforms ( <i>Klebsiella</i> , <i>Citrobacter</i> , <i>Enterobacter</i> ) Spore forming bacteria ( <i>Propionibacterium cyclohexanicum</i> , <i>Clostridium butyricum</i> , <i>Clostridium sporogenes</i> , <i>Alicyclobacillus</i> sp.)	Beverage spoilage
Toxins	Aflatoxins, ochratoxin A, patulin, trichothecenes and zearalenone	Beverage spoilage
Pathogenic species	Bacteria ( <i>Salmonella</i> , <i>Yersinia enterocolitica</i> , <i>Listeria monocytogenes</i> , <i>E. coli</i> ) Viruses (Hepatitis A, norovirus, and rotavirus) Protozoa ( <i>Cryptosporidium parvum</i> )	Foodborne disease

inappropriate for a nonalcoholic drink due to the accumulation of high level of ethanol (as end product of yeasts fermentation) (Stratford, 2006), (ii) some yeasts species degrade preservatives from the beverages, changing the pH of the product, and further the flavor (Stratford et al., 2007.). Species as *Candida davenportii*, *Candida parapsilosis*, or *Debaryomyces* spp. could induce spoilage of the product when the production process goes wrong. The presence of the red aerobic yeasts *Rhodotorula*, *Sporidiobolus*, and *Sporobolomyces* and the black genus *Aureobasidium* indicates a bad hygiene, but usually not determine the spoilage of the beverages (Davenport, 2005).

### 14.3.2 Molds

Molds can survive also in the conditions found in beverages (pH values), but unlike yeasts, molds are strict aerobic species and require oxygen for growth. Spores of *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, and *Fusarium* can survive in beverages and could often be detected in soft drinks. They produce pectinases that change the taste and flavor of the product, toxic compounds and allergens. The most involved mycotoxins in spoilage of beverages are aflatoxins, ochratoxin A, patulin, produced by *Aspergillus*, *Penicillium*, *Fusarium*, or *Alternaria* and trichothecenes and zearalenone produced by *Fusarium* species (Juvonen et al., 2011; Scholte et al., 2004; Swanson, 2011). Heat-resistant molds as *Byssochlamys spectabilis*, *Talaromyces* spp., *Penicillium arenicola*, and *Neosartorya* spp. survive at 90°C (sterilization-pasteurization) determine spoilage of pasteurized juice.

### 14.3.3 Bacteria

Lactic acid bacteria (LAB) are the main bacterial species involved in beverage spoilage: *Lactobacillus paracasei*, *L. brevis*, *L. buchneri*, *L. plantarum*, *L. perolens*, and also *Leuconostoc mesenteroides*, and *Weissella confusa* (Back, 2005). Their metabolism products determine loss of carbonation, an increasing astringency in soft drinks, modification of smell (Lawlor et al., 2009). Another category of bacteria found in beverages are acetic acid bacteria (AAB)—a group of acid-tolerant bacteria that grow at pH 3.0–3.8 and produce some acids (acetic, gluconic, lactic, and succinic acids). Their growth determines flavor changes, package swelling, ropiness, haze, or sediments, especially in case of beverages packaged in PET bottles (oxygen-permeable containers). Coliforms, although acid intolerant species, are able to multiply in beverages with low pH values and produce extracellular polymers being detected in biofilm in plant environments (Kregiel and Rygala, 2010). *Propionibacterium cyclohexanicum*, a

Gram-positive pleomorphic rod, survives in pasteurized beverages. *C. butyricum*, *Clostridium sporogenes*, and *Alicyclobacillus* sp. are other spore-forming bacterial species, succeeding to survive and often associated with the spoilage of soft drinks (Kregiel, 2015).

### 14.3.4 Pathogenic Species

Besides above-mentioned microbial species involved in spoilage of beverages, there were also identified some pathogen species that do not belong to autochthonous microbiota of the fruits. The contamination has fecal origin and the contaminants could survive for different periods of time in juice. The most isolated species from beverage-related outbreaks of foodborne diseases are the EHEC pathotype of *E. coli*, the serotype O157:H7, and various serotypes of *Salmonella*, species that are able to survive up to 48 h in cola drinks (Parish, 2009; Sheth et al., 1988). Other species, as *Yersinia enterocolitica* and *Listeria monocytogenes* survive long periods in different juice concentrates maintained in the freezer and in freshly orange juice (pH 6.3) and moreover, incubated at a low temperature (4°C) usually enhances their survival (Lucero Estrada et al., 2010; Degirmenci et al., 2012). Protozoa and viruses require a host cell for replication, but can survive long time in the environment, as oocysts, for example, and can also be associated with fruit juice-related disease outbreaks. *Cryptosporidium parvum* was isolated from juice and cider. Hepatitis A, norovirus, and rotavirus could potentially transmit diseases via improperly produced beverages (Kregiel, 2015).

## 14.4 Conclusion

In the past years, the consumption of bottled water and soft drinks is preferred to consumption of tap water, the public considering them to be sterile, uncontaminated due to the fact that these products are more processed, and somehow more controlled. But, the risk for health should not be minimized, taking into account the microbial metabolic diversity and versatility, making different species capable to survive, and even multiply in the conditions offered by bottled waters and beverages. On the other hand, the supplementary procedural steps (packaging, bottling, handling, transport) may sometimes increase the risk of microbial contamination of the final product. Therefore, the consumption of bottled waters and beverages is not free of any risks regarding microbial contamination and consequent water or foodborne disease, especially in countries where the legislation and control of these products are not strictly regulated and respected.

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